

Screening of multiple hemoprotein-specific aptamers and their applications for the binding, quantification, and extraction of hemoproteins in a microfluidic system

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ABSTRACT

The blood hemoproteins, albumin, γ -globulin, and fibrinogen, serve as biomarkers for a variety of human diseases, including kidney and hepatorenal syndromes. Therefore, there is a need to quickly and accurately measure their concentrations in blood. Herein, nucleic acid aptamers demonstrating high affinity and specificity toward these hemoproteins were selected via systematic evolution of ligands by exponential enrichment, and their ability to capture their protein targets was assessed with sodium dodecyl sulfate-polyacrylamide gel electrophoresis followed by a tetramethyl benzidine assay. The limits of detection for the hemoproteins were all around $10^{-3}\mu\text{M}$, and dissociation constant values of 131, 639, and 29nM were obtained; capture rates were measured to be 66%, 71%, and 61%, which is likely to be suitable for clinical diagnostics. Furthermore, a multi-layer microfluidic disk system featuring hemoprotein-specific aptamers for depleting hemoproteins was demonstrated. It could be a promising approach to use aptamers to replace conventional antibodies.

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NOMENCLATURE

ΔG	= free energies
3D	= three-dimensional
bp	= base pairs
ddH ₂ O	= double-distilled water
dNTPs	= deoxynucleotide triphosphate
FAM	= fluorescein amidite
HRP	= horseradish peroxidase
K _d	= dissociation constant
kDa	= kilo-Dalton
LOC	= limit of detection
PBS	= phosphate buffered saline
PCR	= polymerase chain reaction
PMMA	= poly (methyl methacrylate)
RPM	= revolution per minute

RT	= room temperature
SDS-PAGE	= sodium dodecyl sulfate-polyacrylamide gel electrophoresis
SELEX	= systemic evolution of ligands by exponential enrichment
ssDNA	= single-stranded DNA
TMB	= tetramethyl benzidine

I. INTRODUCTION

Kidneys have several important functions, including (1) control salt-water balance, (2) remove metabolic wastes from blood and transfer them into urine, (3) carry out gluconeogenesis, and (4) produce enzymes such as erythropoietin and renin.¹ When renal function is reduced to <30%, humans may die.² Chronic glomerulonephritis is a common disease that occurs in about 70% of

those experiencing loss of kidney function.³ Hemodialysis is a procedure whereby blood is removed from the body through an osmotic membrane, semi-purified, and then re-transported back into the body. According to the report from the Center for Disease Control of the Ministry of Health and Welfare of Taiwan,⁴ 0.2%–0.3% of people underwent hemodialysis over the past 10 years, and the number is growing each year.

Although hemodialysis can efficiently remove urinary toxins, ions, and excess water from patients through ultrafiltration through semipermeable membranes,⁵ excessive amounts of hemoproteins (e.g., albumin, γ -globulin, and fibrinogen) may not be removed. This issue may prove lethal since these proteins are involved in kidney/hepatorenal diseases, inflammation, and thrombosis.^{6–8} The total concentration of hemoproteins in plasma is about 60–83 g/l.^{9–10} Plasma also contains plasma proteins, enzymes, antibodies, inorganic salts, lipoproteins, and other cellular nutrients and metabolic related products. The former group features the aforementioned hemoproteins, which form the bulk of all plasma proteins. For instance, 60%–72% of all plasma proteins are albumins, which are produced in the liver and act to maintain a constant plasma colloid osmotic pressure.¹¹ The normal ranges of albumin and globulin in plasma are 3.5–5.5 and 2.5–3.5 g/dl, respectively. Higher ratios of albumin/globulin (>1) are evidence of severe dehydration, shock, hemorrhage, burns, and/or kidney disease.¹² Conversely, low levels of albumin (<3.5 g/dl) are mainly caused by low immunity, abnormal liver function, and impaired kidney function.¹²

Globulins (including α_1 , α_2 , β , and γ -globulins) are synthesized by the mononuclear phagocytic system;¹³ among them, γ -globulins account for the largest quantity of total globulin (about 15% of total plasma protein content). Most human immunoglobulins are γ -globulins. When the concentration of globulin is less than 2.5–3.5 g/dl, it is generally a sign of malnutrition, insufficient protein intake, and/or anemia.¹⁴ Conversely, higher concentrations of globulin are usually associated with abnormal liver function (e.g., cirrhosis)¹⁵ or immune system disorders (such as tumor and giant cell emphysema).¹⁶

Fibrinogen is mainly found in plasma, platelets, and megakaryocytes,¹⁷ and normal concentrations are between 200 and 400 mg/dl.¹⁸ Fibrinogen can be cleaved by thrombin into fibrin to promote blood coagulation.¹⁹ When the level of fibrinogen is too low (<150 mg/dl), coagulation may be compromised; this can occur in individuals experiencing abnormal liver function or blood diseases.²⁰ On the other hand, high levels may be related to inflammation and can cause stroke and cardiovascular diseases.¹⁸

Hemoproteins cannot be removed or added during traditional dialysis. Therefore, it is extremely important to maintain their levels in blood. At present, hemoproteins can be detected by biochemical analysis of turbidity, protein electrophoresis, enzyme-linked immunosorbent assays (ELISA), or flow cytometry.^{21–24} Currently, the specific antibodies are often used to recognize and capture for related hemoproteins in clinical diagnosis and treatment. However, these conventional antibodies are relatively expensive, could be hindered by batch-to-batch variations, and easily exhibit cross-reactions and low specificity. Therefore, alternative approaches had been demonstrated by using single-stranded nucleic acid-based aptamer probes arising as one potential replacement.^{25–26} Aptamer is a single-strand nucleic acid with a

length of 20–80 base pairs (bp) and can form specific three-dimensional (3D) structures depending on its nucleic acid sequences. The 3D structure could interact with specific targets based on hydrogen bonds, van der Waals forces, and hydrophobic interactions. The aptamer has been demonstrated as a next-generation artificial antibody for clinical diagnosis to recognize cancer cells and pathogens.^{27–29} High-specificity and affinity aptamers have been selected from single-stranded DNA (ssDNA) libraries via systemic evolution of ligands by exponential enrichment (SELEX) screening technology.³⁰ Compared with traditional antibodies, aptamers exhibit several advantages, such as (1) they can be chemically synthesized without batch-to-batch variations, (2) they are more relatively stable than antibodies in terms of temperature and humidity, and (3) they are easy to be chemically modified.³¹ Recently, both traditional and on-chip SELEX screening processes have been performed for aptamer selection *in vitro* for different targets.^{32–33} However, many screened aptamers display different 3D structures in blood vs *in vitro*. Up to date, no aptamer has been used and developed for recognizing hemoproteins in clinical applications. We hypothesized that we could devise a microfluidic system capable of screening aptamers from whole blood samples for the consequent binding of the hemoproteins albumin, γ -globulin, and fibrinogen.

Some important hemoproteins, such as albumin, fibrinogen, and γ -globulin, have demonstrated that the amount of these hemoproteins are highly related to physiological conditions or associated with diseases. Therefore, the regulation of these hemoproteins plays an important role for dialysis patients, whose excessive hemoprotein proteins are commonly removed in clinical treatment. However, dialysis was dependent on the molecular size of hemoproteins with no specificity with target proteins. Moreover, relevant antibodies could be used to deplete excessive hemoproteins. However, cross-reactions and relatively high cost are serious issues during treatment. In this study, highly specific aptamers against three hemoprotein were selected by using modified SELEX methods. With this approach, multiple hemoproteins could be captured simultaneously by using the screened aptamers and multi-layered microfluidic disks, which may be promising for the dialysis process in the future. Moreover, a new assay using the screened aptamer was reported, which is also useful during the dialysis process.

II. MATERIALS AND METHODS

A. Aptamer selection for hemoproteins by in multiple selection SELEX

An ssDNA library (5'-GGCAGGAAGACAAACA-N₄₀-TGGT CTGTGGTGCTGT-3') was prepared by Protech Inc. (Taiwan) for selection of hemoprotein-specific aptamers. A novel in multiple selection SELEX process that featured three steps—affinity (positive selection), specificity (negative selection), and in-blood selection—was developed herein [Fig. 1(a)]. The tested target human hemoproteins, such as fibrinogen and γ -globulin, were conjugated to 4.5- μ m magnetic DynabeadsTM (M450 epoxy, 4 \times 10⁸ beads/ml, Invitrogen, USA) (10 μ M stock; purchased from Sigma-Aldrich, USA). Moreover, the 1- μ m magnetic DynabeadsTM (Myone Streptavidin C1, 4 \times 10⁸ beads/ml, Invitrogen, USA) was used to be conjugated with anti-human serum albumin conjugated biotin

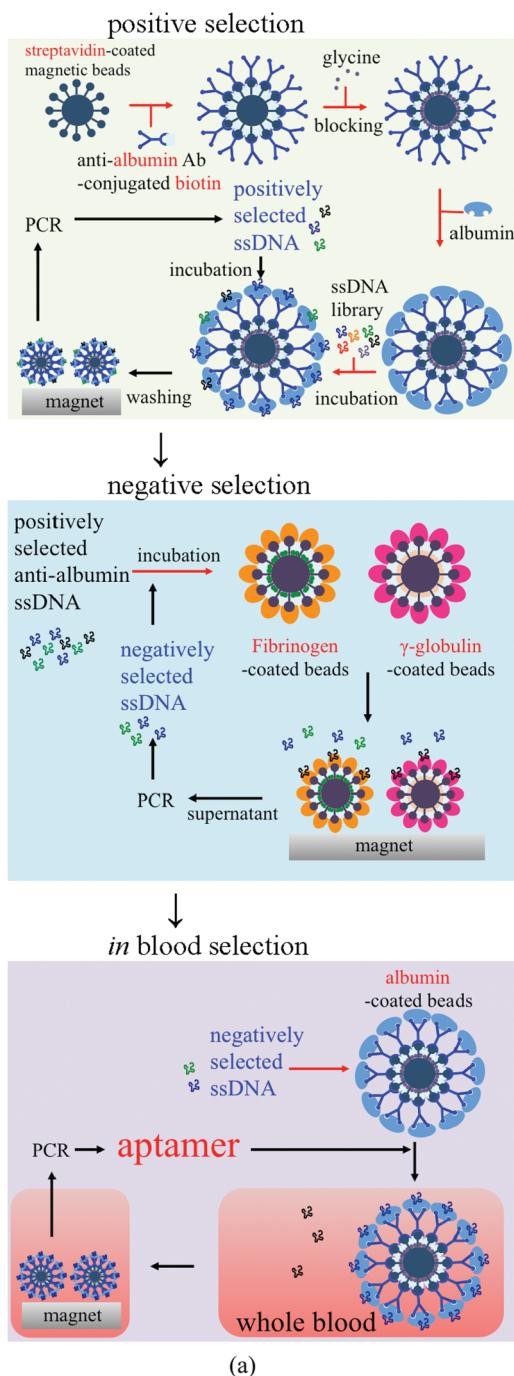


FIG. 1. (a) A schematic illustration of novel, *in* multiple selections of the SELEX process for screening of hemoprotein-specific aptamers, which involved positive selection, negative selection, and in-blood screening. (b) The 3D structural conformations and corresponding free energies of the selected aptamers, as predicted by a bioinformatics website (Kinefold). Albumin-specific aptamer, $\Delta G = -1.4$ Kcal/mole; fibrinogen-specific aptamer, $\Delta G = -9.2$ Kcal/mole; and γ -globulin-specific aptamer, $\Delta G = -5.1$ Kcal/mole.

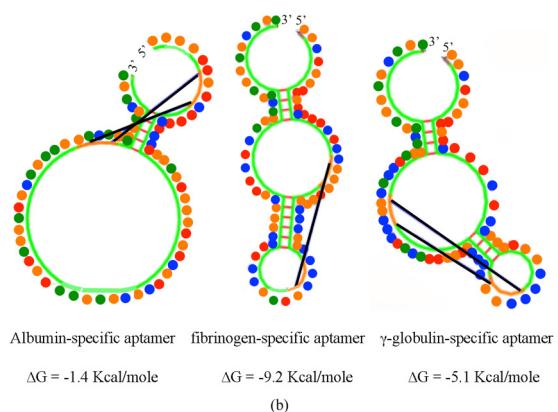


FIG. 1. (Continued.)

(ab24207, Abcam plc., USA). Finally, the human albumin has been conjugated onto the magnetic beads for the following SELEX steps. For positive screening, high affinity ssDNA were adhered to the target hemoproteins surface-coated on the magnetic beads. Once the beads were collected under a magnet, they were washed with 1× phosphate buffered saline (PBS, 137mM NaCl, 2.7mM KCl, 100mM Na₂HPO₄, and 1.8mM KH₂PO₄; pH 7.4) to remove unbound ssDNA. The captured ssDNAs were then amplified by polymerase chain reaction (PCR) with forward (5'-GGCA GGAAGACAAACA-3') and reverse (5'-ACAGCACCACAGA CCA-3') primers (0.5 μ l of 10 μ M), and a mastermix composed of 0.5 μ l of 10mM deoxynucleotide triphosphate (dNTPs, Promega, USA), 2 μ l of 10x SuperMix buffer (GeneDireX, Taiwan), 0.5 μ l of Taq DNA polymerase (5 U/ μ l, GeneDireX), 6 μ l of double-distilled water (ddH₂O), and 10 μ l of the collected ssDNA. The PCR thermocycling protocol was as follows: 95 °C for 5 min, followed by 30 cycles of 95 °C for 20 s, 56 °C for 15 s, and 72 °C for 15 s. Then, the amplified PCR products were denatured (95 °C for 5 min) and stored at 4 °C for generation of single-stranded nucleic acids for the next round of SELEX. The PCR and gel electrophoresis were performed to analyze whether the 72-bp products were amplified to confirm the screening process after each SELEX round.

After six rounds, positively selected ssDNAs that exhibited high affinity toward one of the target hemoproteins were incubated with non-target hemoproteins surface-coated on magnetic beads for negative selection. Then, non-specific or low-affinity ssDNAs bound with target hemoproteins were captured onto the magnetic beads surface-coated with non-target hemoproteins. After washing under a magnetic field, these low-affinity and specificity ssDNAs were excluded from the aptamer candidates. Therefore, only ssDNAs with high affinity and specificity remained in the supernatant after negative selection. After six rounds of negative selection, the collected, high affinity and specificity ssDNAs were incubated with magnetic beads surface-coated with the target hemoproteins in blood and washed with 1× PBS to ensure that the final aptamer candidates would be functional in clinical blood samples. Note that SELEX screening in plasma could improve the affinity and specificity of the aptamers.

specificity of the screened aptamer in blood.³⁴ Since the 3D conformation of the aptamers could be affected by temperature, pH, or ion species/concentrations,^{35–37} the collected, negatively selected ssDNAs were incubated with the corresponding hemoproteins surface-coated on the magnetic beads in blood.

After all SELEX screening steps, each hemoprotein-specific aptamer candidate was cloned with the TOPO® TA cloning kit (Invitrogen), and 30 clones were sequenced. The 3D structures of the most represented aptamers in the clone library were predicted by Mfold and KineFold (<http://unafold.rna.albany.edu/?q=mfold> and <http://kinifold.curie.fr/>, respectively). The ideal aptamers were not only highly specific, but they also possessed (1) more complex 3D structures (i.e., more loops and stem-loops) and (2) lower free energies.

B. Detection of hemoproteins and specificity tests of hemoprotein-specific aptamers

The selected aptamers (10 µl of 10 µM) were conjugated to magnetic beads (10⁸ beads/ml) and incubated with three target hemoproteins (200, 100, 50, or 25 µg in 100 µl of 1× PBS) at 20 revolution per minute (RPM) for 30 min at room temperature (RT; 25 °C). After three PBS washing processes, all bead-aptamer-target protein complexes were collected by applying an external magnetic field. These complexes were pretreated by boiling for 20 min and subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). After the boiling step and SDS treatment, the target protein was denatured and released from beads to confirm whether the screened aptamers could capture the corresponding target hemoprotein. The 6% polyacrylamide gel was prepared according to the protocol provided by BioRad (USA). The tested samples were loaded, and the gel was electrophoresed in 1× SDS-PAGE running buffer at 100 V for 1 h. Then, the acrylamide gel was stained with 2% Coomassie blue (1 g of Coomassie Brilliant Blue-R-250, in 150 ml of methanol, 50 ml of glacial acetic acid, and 300 ml of ddH₂O) for 2 h at RT. Next, the staining solution was decanted, and the gel was destained in 200 ml of methanol, 100 ml of glacial acetic acid, and 700 ml of ddH₂O for 2 h at RT.

A new assay to measure the concentration of hemoproteins was further developed with the screened aptamers. The tetramethyl benzidine (TMB) assay was further adopted to measure the concentration of these hemoproteins. First, 10 µl of selected aptamers (10 µM) were conjugated to magnetic beads (10⁸ beads/ml) and incubated with tenfold serially diluted proteins (10¹ to 10⁻³ µM) at 20 RPM for 30 min at RT. Then, biotin-labeled 2nd specific aptamers were incubated with the above complexes. After washing with ddH₂O, the streptavidin-horseradish peroxidase (HRP) was used to recognize the aptamer-target protein-2nd biotin-labeled

aptamer complexes. After washing steps, TMB was further added, thus resulting in blue color upon interacting with HRP-conjugated complexes. The limit of detection (LOD) for fibrinogen was determined in both ddH₂O and blood with hemoprotein-specific aptamers followed by a TMB assay. To verify specificity of the selected aptamers, 10 µl of albumin (1 µM) and γ-globulin (1 µM) were used as negative controls while testing fibrinogen-specific aptamer-coated beads (10 µl of 10⁷, 5 × 10⁷, or 10⁸ beads).

C. Microfabrication of the multi-layer microfluidic disk system featuring hemoprotein-specific aptamers

A computer-numerical-control machining process (EGX-400, Roland DGA, Japan) was used to prepare a novel, single-layer hemoprotein-capturing disk on poly (methyl methacrylate) (PMMA) plates; this disk featured a resin reservoir and a cover. Aptamer-immobilized resins were assembled on one side of the reservoir for the capture of target hemoproteins, while the reservoir on the opposite side contained supplemental hemoproteins. A hollow columnar hold was fabricated by an Mbot 3D CUBE II printer (Armsource, Taiwan). A middle column was printed such that disks could be stacked in series during hemodialysis. The microfluidic disk and the bottom layer were also equipped with holes for injection and removal of blood.

D. Aptamers immobilized onto carboxylic acid-modified resins and hemoprotein capture rate tests

The hemoprotein-specific aptamers were first bound to amide groups and immobilized onto carboxylic acid-modified resins (CarboxyLink™ coupling resins, Thermo Fisher Scientific) such that they would capture hemoproteins. First, 100 µl of resins was mixed with 200 µl of water and then centrifuged at 1000 × g for 2 min at RT (×3 repetitions). Then, 100 µg of the selected aptamer (in 0.1M imidazole, pH 6.0) and 120 µl of 0.1M imidazole were added and mixed thoroughly with the resins for 3 h at RT. After spinning thrice at 1000 × g, 100 µl of ddH₂O was added to aptamer-modified resins prior to use.

The capacity of the aptamer-immobilized resins to capture each target hemoprotein was further explored. To exclude interferent factors from blood for the capture assay, 100 µl of purified proteins (1 mg/ml) in 1× PBS buffer was injected into the single-layer microfluidic disk system at RT. After 10 min, the protein solution eluted from the blood outlet port was collected and the final amount of protein was measured with a Bradford protein assay.³⁸ The capture rate of albumin-specific aptamers was then calculated as the input quantity minus the eluted quantity divided by the input quantity.

TABLE I. Sequences of screened aptamers specific to three target hemoproteins.

Aptamer target	sequence (5'→3')
Albumin	ACAGCACCAACAGACCAACAGCTACCATAACACTCGCGTCGTCCCTCACCTGGTGGTCTTCCTGCC
Fibrinogen	GGCAGGAAGACAACACCGGGAGGGCCCCGGATGCCAACGGGGGAACACTGGTCTGTTGCTGT
γ-Globulin	GGCAGGAAGACAACACCGGGAGGGCCCCGGAGGAATCTACCCGGCTCCATGGTCTGTTGCTGT

E. Dissociation constants of the screened aptamers

Binding affinity is the strength of the binding interaction between a single biomolecule (e.g., albumin, fibrinogen, and γ -globulin) and its ligand/binding site (e.g., aptamer). Binding affinity is typically measured by the equilibrium dissociation constant (K_d), which is used to evaluate the strength of bimolecular interactions. The smaller the K_d value, the greater the binding affinity of the ligand for its target. The binding affinities of the screened aptamers were determined by measuring the dissociation constants (K_d s) of each aptamer for its target hemoprotein. Fluorescein amidite (FAM)-labeled hemoprotein-specific aptamers (100 or 1000 nM) were incubated with 100 μ l of each target hemoprotein (1 μ g/ μ l) at concentrations at RT for 30 min. Afterward, fluorescence intensity was measured by a BD AccuriTM C6 Plus system (USA), and the ligand binding analysis function of Prism software ver. 5.0 (GraphPad, USA) was used to determine the ratio of fluorescent aptamer-bound hemoprotein and the apparent K_d of the screened aptamers according to the fluorescence intensity at both concentrations.³⁹

III. RESULTS AND DISCUSSION

A. Characterization of the hemoprotein-specific aptamers

After multiple selection of SELEX, all collected aptamers were cloned and sequenced (Table I). The 3D conformations of the albumin, fibrinogen, and γ -globulin-targeting aptamers were modeled by using the bioinformatics web server [Fig. 1(b)]. The bold line in the figure indicated intermolecular hydrogen bonds within the predicated aptamer. Moreover, their free energies (ΔG) were predicted to be -1.4 , -9.2 , and -5.1 Kcal/mole. Although the free energy of the former is relatively high, it nevertheless exhibited a significant secondary structure. Moreover, it was sequenced in 26 of the 30 screened clones (87%), indicating that it was most likely to bind to its hemoprotein target. Similarly, the other two aptamers were also proportionally dominant among the clone sequences (data not shown).

B. Multi-layer microfluidic disk system

A single-layer microfluidic disk was assembled, and the diameters of the reservoir and upper cover were 2.2 and 2.6 cm, respectively [Fig. 2(a)]. The assembled disk possessed a 0.5-cm-diameter hole in the middle so that it could be inserted into the center of a disk holder [Figs. 2(a) and 2(c)]. The number of aptamer-coated resin disks could be increased, depending on the hemoprotein needed. With this system, three hemoprotein-specific aptamers could be used simultaneously to capture their target hemoproteins.

C. Affinity and specificity of hemoprotein-specific aptamers

The albumin-, fibrinogen-, and γ -globulin-specific aptamers could bind (1) albumin (67 kDa), (2) the β (56 kDa) and γ chains (47 kDa) of fibrinogen, and (3) the heavy chain (55–60 kDa) of γ -globulin, respectively [Figs. 3(a)–3(c)], and the minimum

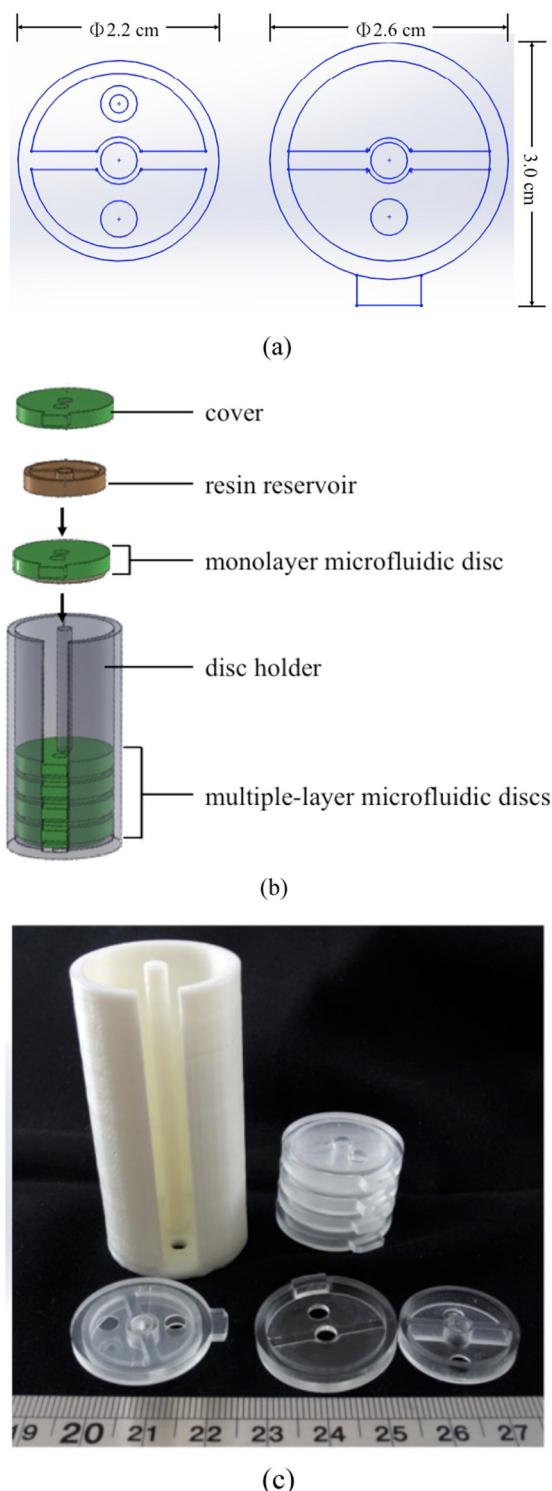
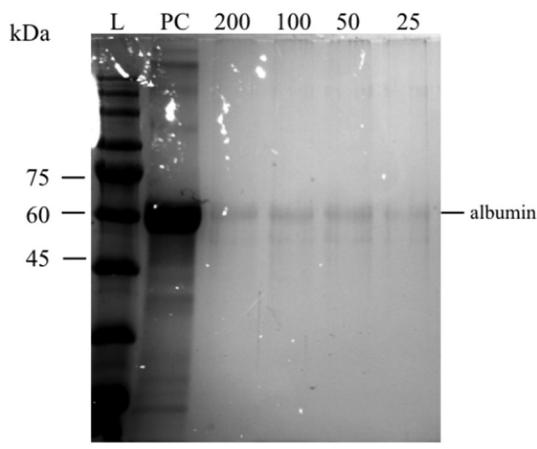
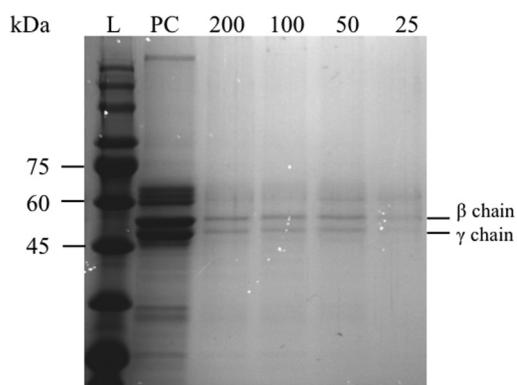


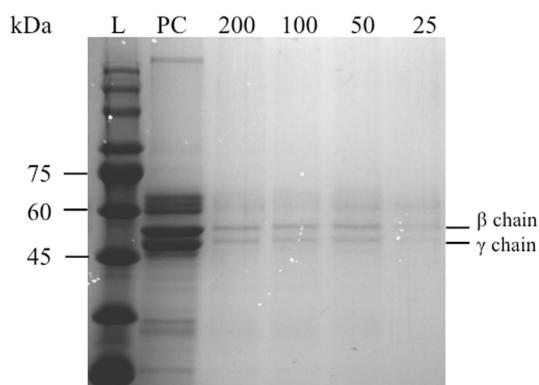
FIG. 2. (a) A schematic diagram of a single microfluidic disk. (b) Exposed view and (c) photograph of the multi-layer microfluidic disk system.



(a)



(b)



(C)

FIG. 3. The selected hemoprotein-specific aptamers were coated onto magnetic beads and incubated with the target hemoproteins at concentrations of 200, 100, 50, or 25 μg in 100 μl of 1× PBS. Coomassie blue stain results showing the binding ability of albumin-specific (a), fibrinogen-specific (b), and γ-globulin-specific (c) aptamers with SDS-PAGE.

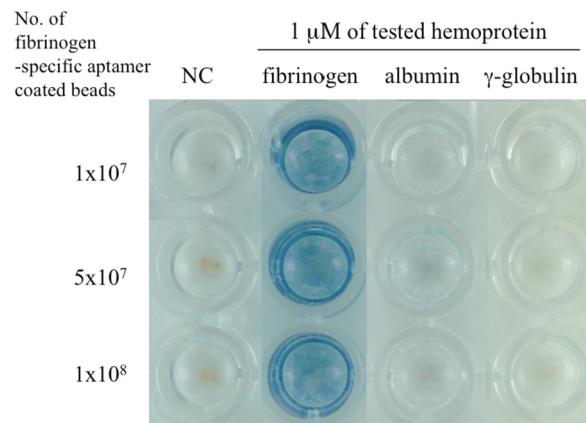
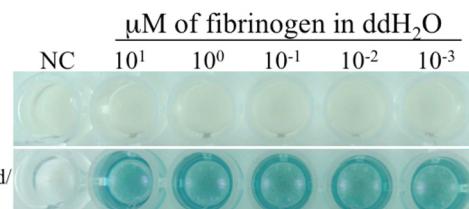
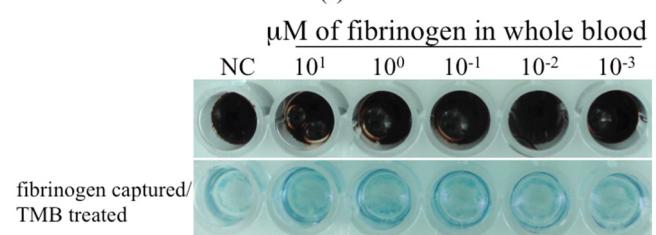


FIG. 4. The specificity tests of the fibrinogen-specific aptamers with 1 μM of fibrinogen, albumin, and γ-globulin. Biotin-labeled 2nd specific aptamer-conjugated HRP and TMB assays were used, and blue color signified a positive result; the fibrinogen-specific aptamer-coated beads (10⁷, 5 × 10⁷, and 10⁸ beads) could only bind 1 μM of fibrinogen.

amounts of target proteins that could be visualized with Coomassie blue staining were 25, 25, and 50 μg, respectively. These LODs are lower than those obtained with the blood samples discussed above. In terms of specificity of the TMB assay, the fibrinogen aptamer-coated beads (10⁷ beads) could specifically bind 1 μM of fibrinogen, neither recognizing albumin nor γ-globulin [Fig. 4]. Similarly, the screened albumin- and γ-globulin-specific aptamers exhibited



(a)



(b)

FIG. 5. The limit of detection of the fibrinogen-specific aptamer in ddH₂O (a) and whole blood (b). The upper row shows the pre-aptamer treatment state, while the images in the bottom row showed the results of the TMB assay.

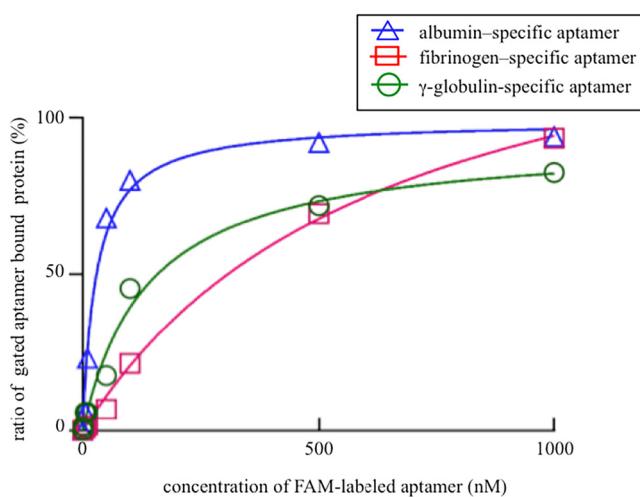


FIG. 6. Measuring the dissociation rates and dissociation constants (K_d) of the aptamer-hemoprotein complexes using FAM-labeled aptamers.

similar specificity to their protein targets. The images in the upper and lower rows of Fig. 5 show the pre- and post-aptamer incubation steps, respectively, and the LODs for fibrinogen in ddH₂O and blood were both $10^{-3}\mu\text{M}$ [Figs. 5(a) and 5(b)], well below the normal blood concentrations ($\sim 10\text{--}20\text{mM}$). Therefore, the specific aptamer could be used to directly isolate this protein from blood,

which may prove advantageous for clinical applications, as well as the bulk preparation of fibrinogen for hemodialysis.

The K_d s of the hemoprotein-specific aptamers were further investigated via FAM-labeling [Fig. 6], and the hemoprotein-specific aptamers showed high binding affinities with relatively low K_d : 130.7 ± 20.6 , 639.0 ± 88.6 , and $28.7 \pm 4.8\text{nM}$ for albumin, fibrinogen, and γ -globulin, respectively. The K_d s of aptamers for other hemoproteins, such as β_2 -microglobulin, lipopolysaccharide binding protein, and thrombin, are far higher ($1\text{--}1000\mu\text{M}$),^{40–42} indicating that the screened aptamers exhibited high affinity.

In order to demonstrate the capacity of the selected aptamers to capture the target hemoproteins, they were conjugated with carboxylic-labeled resins and assembled into a multi-layer microfluidic disk system [Figs. 7(a) and 7(b)]. Red ink was used as an indicator for permeability tests, and the cross-sectional and top views of different disks were observed; image analysis revealed that samples could steadily pass through the resin-based tri-layer microfluidic disk device within 10 min [Figs. 7(c) and 7(d)]. After verifying this, $10\mu\text{M}$ of each aptamer was coated onto carboxyl resins to measure the capture rates, and values for albumin, fibrinogen, and γ -globulin were measured to be $66.1 \pm 4.1\%$, $70.7 \pm 1.7\%$, and $60.8 \pm 2.2\%$, respectively. In contrast, the aptamers had low capture rates ($\sim 10\%$) for non-target proteins (Table II). To further improve capture rates (e.g., for hemodialysis), pneumatic pumps could be used to enhance fluid transport, or additional aptamer-coated resin disks could be incorporated; less dense resins could also be exploited to improve the permeability rate.

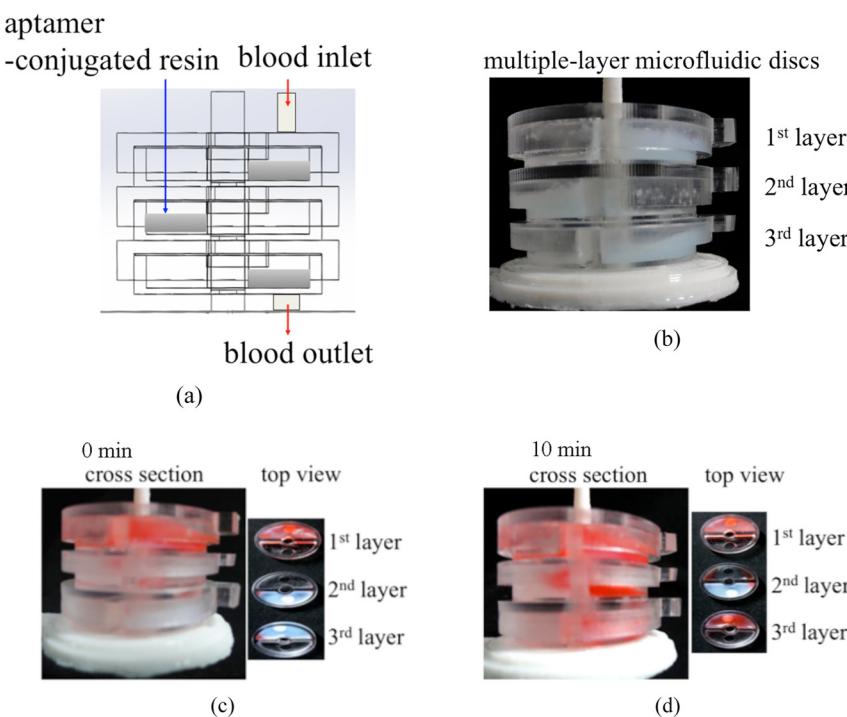


FIG. 7. The permeability test for screened hemoprotein-specific aptamers immobilized onto carboxylic-labeled resins and assembled into a tri-layer microfluidic disk system. The schematic diagram of stacked microfluidic disks (a) and a photograph of the hemoprotein-specific aptamer-coated resins within the reservoir chamber (b). Red ink was used as an indicator of permeability. The cross-sectional and top views of different disks were observed after 0 (c) and 10 (d) min.

TABLE II. Capture rates of hemoprotein-specific aptamers (top row) with their target proteins (left-most column). Error terms represent standard error ($n = 3$).

	Anti-albumin (%)	Anti-fibrinogen (%)	Anti- γ -globulin (%)
Albumin	66.1 ± 4.1	12.8 ± 2.3	11.5 ± 3.2
Fibrinogen	15.9 ± 4.3	70.7 ± 1.7	11.0 ± 1.2
Globulin	12.0 ± 1.6	17.0 ± 0.8	60.8 ± 2.2

III. CONCLUSIONS

This study represents the first time that SELEX has been carried in blood for screening of multiple hemoproteins-specific aptamers; unlike other hemodialysis approaches (e.g., charcoal-binding of albumin⁴³ and glycoprotein IIb-IIIa complex-binding of fibrinogen),⁴⁴ the aptamers unveiled are relatively cheap to synthesize. Experimental results showed that the screened aptamers could be used for binding, quantification, and extraction of hemoproteins. Aptamers capable of recognizing other hemoproteins (e.g., macroglobulins) could likewise be identified by using the similar approach. The multi-layer microfluidic disk system could even be used for hemodialysis.

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